

LXVIII. THE CATALYTIC ACTION OF CUPRIC SALTS IN PROMOTING THE OXIDATION OF FATTY ACIDS BY HYDROGEN PEROXIDE.

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(Received June 4th, 1929.)

DURING the last few years experiments on the oxidation of the higher fatty acids by means of hydrogen peroxide in the presence of ferrous iron have been carried out in this laboratory by C. G. Daubney; the results however were found to be exceedingly irregular. Under conditions which were apparently similar, in some experiments oleic acid was rapidly attacked and largely broken down to carbonic, formic, acetic and succinic acids; in other experiments hardly any action took place. The alteration in the conditions, however, which so greatly increased the oxidation eluded discovery. In a further attempt to solve this problem, the present authors endeavoured to investigate the effect of the addition of small quantities of impurities to the reaction mixture.

The conditions of the original experiments were as follows. Into a large flask were introduced 2.5 g. oleic acid, 10.6 cc. *N* sodium or ammonium hydroxide and 125 cc. distilled water: when the oleic acid was dissolved, 15 cc. of a solution containing 0.613 g. ammonium ferrous sulphate and 350 cc. hydrogen peroxide solution (20 vols.) were added to the soap solution. The flask fitted with a reflux condenser was left for 2 hours in a bath at 60°, the outlet being connected to an apparatus for the absorption of CO₂. At the end of this time, 2 cc. concentrated sulphuric acid were added and nitrogen was blown through the apparatus for a further 2 hours. Any insoluble acid was then filtered off and the liquid steam-distilled.

We first investigated the effect of the addition of small amounts respectively of nickel, manganese, zinc and copper salts which were added to the mixture at the same time as the ferrous salts. It was at once evident that the reaction was influenced by the addition of the copper salt but no certain effect was noticeable when nickel, zinc or manganese salt was added. Next we tried the effect of replacing the ferrous salt by an equivalent amount of cupric salt and we found that consistent results were obtained. The oxidation was very much more marked than in the "inactive" experiments with the ferrous salt, but the proportion of volatile acid was never quite as great as in the "active" iron experiments. Since we had now found conditions which were always repeatable, and since under the influence of the cupric salt at 60° in the presence of excess of hydrogen peroxide oleic acid may be largely

broken down into carbonic, formic, acetic and succinic acids, we decided to investigate this reaction quantitatively. We do not, however, think that the condition in the original experiments with ferrous salts which determined their activity was the presence of copper salts.

THE INFLUENCE OF COPPER SALTS IN PROMOTING OXIDATION.

A study of the literature showed that the action of cupric salts in promoting the oxidation of tincture of guaiacum in the presence of hydrogen peroxide had been frequently noted and that one or two other instances of their action had been observed. Bourquelot and Bougault [1897] first recorded that in the presence of copper salts tincture of guaiacum is coloured blue, just as it is by the action of oxidising ferments. Here the oxygen necessary for the oxidation is derived from the air, and the copper salt behaves as an oxidase. Thus, if to a 2 % solution of quinone two or three drops of a 1 % solution of copper acetate are added, the characteristic blue colour is produced slowly in the cold, more rapidly on heating. The presence of oxygen is essential and the colour is not produced *in vacuo*. Labat [1908] recorded that an intense red colour was produced with the Kastle-Meyer reagent (an alkaline solution of phenolphthalein decolorised by boiling with zinc dust) in the presence of hydrogen peroxide to which a drop of a 2 % solution of copper sulphate in glycerol had been added. Mueller [1918] again confirmed the observations of Bourquelot and Bougault and drew attention to the behaviour of cupric salts both as oxidases, the supply of oxygen being obtained from the air, and as peroxidases, hydrogen peroxide acting as the source of the oxygen used in the reaction. Mueller suggested that the cupric salt reacts with the hydrogen peroxide, one oxygen atom of the peroxide entering into combination with the partial valencies of the copper and that this unstable compound then reacts with the oxidisable compound.

Thomas and Carpentier [1921] repeated Labat's observation and showed that the Kastle-Meyer reagent is extraordinarily sensitive to the action of copper salts, the colour being perceptible with a solution of copper containing only one part in a hundred million; indeed the distilled water found in most laboratories where copper stills are used for its preparation was found to be active. The close resemblance of this action to that of a peroxidase was again stressed by these authors, who also made the interesting observation that the lactate and tartrate of copper were inactive, but that the acetate and butyrate were active, behaving like the mineral salts. Aloy and Valdiguié [1923] extended these observations and brought forward evidence in support of the view that the intermediate substance concerned in this reaction is an unstable cupric peroxide: this substance they prepared by the method originally described by Thénard and also by the addition of a solution of hydrogen peroxide to one of ammoniacal copper sulphate, and they found that the peroxide behaved as a peroxidase. The activation of oxygen by metallic copper was studied by Wieland [1923]; in his experiments various organic

acids, acetic, pyruvic, succinic, malic, benzoic, oxalic and lactic, were oxidised with the production of carbonic acid, the acids being shaken with oxygen in the presence of metallic copper. He found that the reaction only took place in acid solution and that when the metal had dissolved in the acid oxidation was at an end. He found a similar effect when cuprous salts were added in the presence of hydrogen peroxide and here again the effect was only produced in acid solution and came to an end when the cuprous salt was converted to the cupric state. He says "Vor allem aber büsst das peroxydische oder höherwertige Kupfer nach Abgabe eines Sauerstoffäquivalentes als Cuprisalz sein Oxydationsvermögen ein."

With regard to this we can only say that under the conditions of our experiments the cupric salts are certainly the active agents. Since, as far as our investigations go, the presence of an acid group in the molecule seems essential in order that oxidation may take place, it seems to us possible that the copper enters into the molecule of the oxidisable acid, forming a salt, and that the partial valencies of the copper then unite with the oxygen of the hydrogen peroxide as Mueller suggested, forming an unstable intermediate compound.

The close analogy of the action of cupric salts with that of the peroxidases made us hopeful that the oxidation of the higher fatty acids by means of hydrogen peroxide in the presence of cupric salts might present a closer analogy with the physiological process than oxidation by any other known laboratory method. When the higher fatty acids are acted upon by hydrogen peroxide in the absence of a catalyst they are very little attacked and therefore it has been difficult to establish the nature of the reaction. We decided therefore first to work out the method on the simpler fatty acids and their oxygenated derivatives and later to apply the method to a comparison of the oxidation of palmitic, oleic and stearic acids.

Preliminary experiments on the oxidation of oleic acid indicated that among the products of the reaction were acetic and succinic acids. The behaviour of these acids under the same conditions of oxidation was therefore examined and the reaction extended to other acids which might conceivably occur as intermediate products. Succinic acid was chosen for the investigation of the conditions of the reaction.

Influence of the reaction of the medium. In alkaline or neutral solution very little oxidation of succinic acid occurs; favourable results were obtained when the acidity of the mixture corresponded to 0.010 to 0.020 *N*. It seems essential that the reaction shall be acid but small changes in the hydrogen ion concentration appear to exert little influence.

Method.

2.5 g. of the acid to be oxidised were placed in a large flask and the calculated quantity of *N* NaOH solution was added, in most cases sufficient to make the whole reaction mixture equivalent to an acidity of 0.015 to

0.020 *N*. A measured quantity of a 0.73 % solution of crystalline cupric sulphate and 350 cc. of a previously neutralised solution of hydrogen peroxide (20 vols.) were then added, the latter reagent being present therefore in large excess. The mixture was left in a bath at 60° for a stated time, the outlet of the flask being connected with an absorption apparatus containing a known amount of a standard baryta solution: 2 cc. concentrated sulphuric acid were added to the contents of the flask and nitrogen was blown through for 1½ hours, the bath being meanwhile allowed to cool. When the oxidisable acid is insoluble in water, the addition of the sulphuric acid precipitates any insoluble acid and so prevents the further action of the peroxide. In the case of soluble acids, however, the effect of the hydrogen peroxide on the acid continues though at a lower temperature and in a much more acid medium during the time in which the carbonic acid is being blown off.

Table I.

(a) during time of experiment;

(b) during subsequent steam-distillation at 100°.

Succinic acid g.	Time of exp. hr.	Reaction of mixture	Atomic proportion Cu to 1 mol. succinic acid	Percentage of original succinic acid appearing as			
				CO ₂		Formic	Acetic
				(a)	(b)		
<i>Influence of reaction of medium:</i>							
		Alkali					
2.5	2	0.054 N	0.000	4.0	—	—	—
"	1	"	0.077	0.6	—	—	—
"	0.5	"	0.077	0.8	—	—	—
		Acid					
"	1	0.015 N	0.000	7.1	37.1	—	—
"	1	"	0.077	45.2	6.0	—	—
"	0.5	"	0.077	41.2	8.0	3.9	26.5
<i>Influence of proportion of copper:</i>							
		Acid					
2.5	0.5	0.012 N	0.000	Trace	—	—	—
"	"	0.015 N	0.026	9.4	—	—	—
"	"	0.018 N	0.052	28.8	15.0	5.0	22.0*
"	"	0.020 N	0.078	53.4	5.0	2.0	17.6*
<i>Influence of time of experiment:</i>							
		Acid					
2.5	0.5	0.014 N	0.026	9.7	—	—	—
"	1	"	"	29.6	—	—	—
"	1.5	"	"	48.9	—	—	—
"	2	"	"	56.9	—	—	—

* In these experiments the hydrogen peroxide was removed before the steam-distillation.

The acid mixture is then filtered. In the earlier experiments the solution was then steam-distilled, but in this case the excess of hydrogen peroxide continues to act at a higher temperature on any products in the solution. In the later experiments, therefore, before carrying out the steam-distillation, the excess of hydrogen peroxide was destroyed by making the solution alkaline, adding to it finely powdered charcoal and allowing it to stand overnight.

The next morning the filtered solution was again acidified and steam-distilled, the distillate collected, any emergent gas being passed through a standard baryta solution. The distillate was neutralised with a known amount of *N* NaOH solution and evaporated to dryness. The amount of formate in the sodium salts of the volatile acids was estimated, and the molecular weight determined. In most cases the acid is completely soluble and appears to consist almost entirely of acetic and formic acids. Aldehyde was usually present and was estimated in an aliquot part of the original distillate. The results of the experiments with succinic acid which are collected in Table I show (1) that the addition of the cupric salt is effective in acid but not in alkaline solution; (2) that after the mixture has stood for half an hour at 60°, about 75 % of the succinic acid has been decomposed into carbon dioxide, formic acid and a notable proportion of acetic acid.

Comparison of the relative effect of ferrous, cupric and cuprous salts.

Neither in acid nor in alkaline solution did we find that ferrous salts influenced the oxidation of succinic acid under the conditions of our experiments. The amount of oxidation was here measured by the percentage of the original acid appearing as CO₂ during the time of the experiment. It will be seen that the results of the experiments in which iron was present were similar to those in which no metallic salt was added. The possibility that intermediate products of oxidation were present was not however excluded.

Table II. *Showing the comparative effect of cupric and ferrous salts on the oxidation of succinic acid by hydrogen peroxide.*

Succinic acid g.	Time of exp. hr.	Reaction of mixture (acid)	Metal added	Percentage of original succinic acid converted to CO ₂
2.5	1	0.022 <i>N</i>	—	7.2
"	0.5	0.014 <i>N</i>	Fe	6.0
"	0.5	"	Fe	8.8
"	1	"	Fe	5.2
"	1	"	Fe	4.4
"	0.5	"	Cu	41.2
"	1	"	Cu	50.4
"	1	"	Cu	46.4
"	1	"	Cu	54.0
"	1	"	Cu	45.2

0.078 of an atomic proportion of Cu⁺ or Fe⁺ was taken in each case.

The substitution of cupric chloride or cuprous chloride for cupric sulphate gave similar results. The action of the cuprous chloride was rather slower than that of the cupric compound. The effect of the same salts in promoting the decomposition of the hydrogen peroxide was then studied, the decomposition being measured by the volume of oxygen evolved in the same time under similar conditions at 60°.

70 cc. hydrogen peroxide were neutralised with 1.4 cc. *N* NaOH, 23.6 cc. water and 2 cc. *N* acid added and equivalent amounts of cupric and

cuprous chloride and of ferrous sulphate respectively. The relative amounts of oxygen liberated in the same period of time when measured under similar conditions were respectively 910 cc. (CuCl_2), 610 cc. (CuCl) and 215 cc. (FeSO_4). The control in the absence of metallic salts liberated 160 cc.

It appears therefore that cuprous salts are more effective in bringing about the decomposition of hydrogen peroxide but that cupric salts are more active in catalysing the oxidation of the aliphatic acids by hydrogen peroxide.

We then investigated the effect of the same oxidising agent on some of the acids which might possibly occur as oxidation products of succinic acid and the results which we obtained are set forth in the following table (Table III). In each case the reaction of the mixture was 0.014 *N* acid and 0.078 of an atomic proportion of copper was present for each molecular proportion of the acid to be oxidised: the conditions of the experiments were similar to those already described. The action was estimated by the weight of carbonic acid evolved during the time of the experiment and during the subsequent period in which the carbonic acid is being blown off from the solution. It is possible that, in the absence of the cupric salts, oxidation products containing the same number of carbon atoms may have been produced. We did not however obtain any evidence of this.

Table III.

Acid	g.	Time of exp. hr.	H_2O_2 and Cu		H_2O_2 alone	
			Weight CO_2 g.	Acid converted to CO_2 %	Weight CO_2 g.	Acid converted to CO_2 %
Succinic	2.5	0.5	1.54	41.2	—	Trace
		1	1.68	45.2	—	7.1
			2.01	54.0	—	—
			1.58	42.4	0.04	1.5
			1.87	50.2	—	—
			1.65	44.4	0.006	0.02
Malic	1.0	1	0.77	58.8	0.03	2.3
Tartaric	2.5	0.5	>1.68	>57.3	{ CO_2 evolved too rapidly for complete absorption	
		0.75	>1.93	>65.8		
	1.0	0.5	1.18	100	0.0	0.0
		1	1.18	100	0.0	0.0
Dihydroxymaleic	1.0	1	0.76	64.0	0.29	24.45
	"	1.25	0.72	60.2	0.34	28.60
Pyruvic	1.0	1	0.90	60.00	0.31	21.00
"	"	—	0.85	56.67	—	—
Lactic	1.0	1	0.63	43.3	0.002	0.14
"	"	—	0.56	38.0	—	—
Malonic	1.0	1	0.02	1.8	—	—
	"	1.25	0.48	38.5	—	—
	"	2	0.43	34.6	0.01	0.8
Acetic	2.5	1	1.22	33.3	—	—
	1.0	1	0.47	32.3	0.007	0.4

When tartaric acid is oxidised by means of hydrogen peroxide in the presence of ferrous iron, dihydroxymaleic acid is the first product of oxidation [Fenton, 1894, 1896]. Since, however, in the presence of cupric salts, dihydroxy-

maleic acid is much less readily oxidised to carbon dioxide than is tartaric acid, the evidence available does not support the view that, in the presence of cupric salts, tartaric acid is first converted to dihydroxymaleic acid. Malonic acid showed a curious period of lag before action began in three of our experiments, during the first hour there was practically no evolution of carbonic acid, then in the next 15 minutes a rapid evolution of gas took place. The notable proportion of acetic acid formed during the oxidation of succinic acid was interesting, and the fact that acetic acid itself was also converted to carbonic acid, one-third of the amount taken being completely oxidised to carbonic acid in an hour, was also noteworthy.

In the absence of the cupric salt only two of the acids taken were oxidised by the action of the hydrogen peroxide under the conditions of our experiments. These were pyruvic and dihydroxymaleic acids, but the evolution of carbon dioxide is much greater in the presence of the copper salt.

Similar experiments were carried out using alcohol, acetone and glycol as the materials to be oxidised, but in no case was there any evidence of oxidising action taking place. The addition of dilute sulphuric acid to the mixture produced no effect, and the action of the cupric salt seems only effective when the substance to be oxidised is an acid and the experiment is carried out when a certain proportion of the free organic acid is present.

The oxidation of the higher fatty acids is at present under investigation, but as the method seems to be of general application for the oxidation of the aliphatic acids, we desire to record these preliminary results.

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